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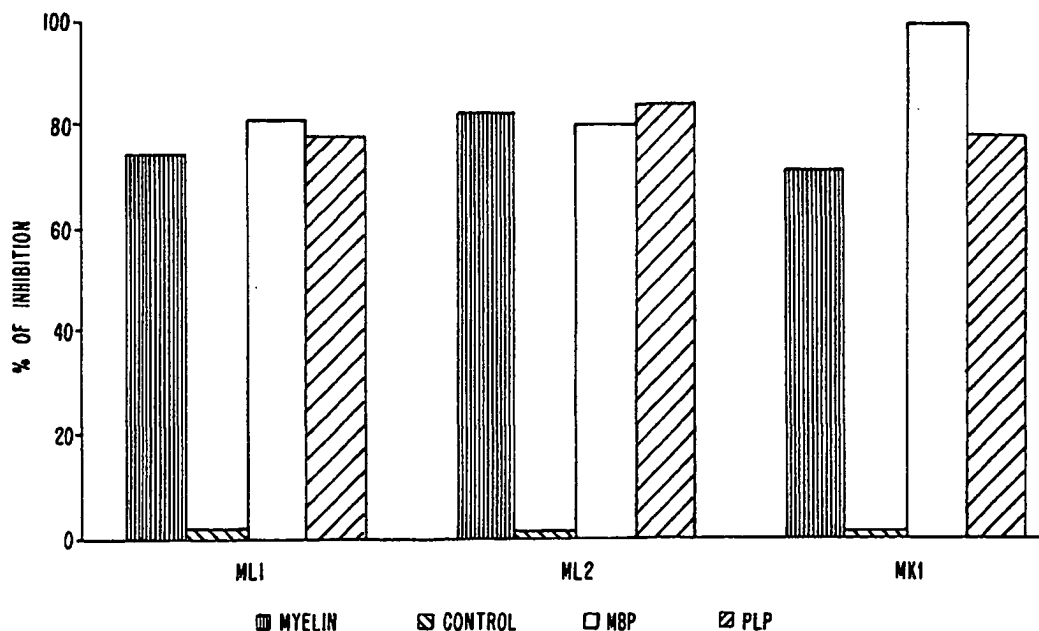
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(54) Title: T-CELL VACCINATION FOR THE TREATMENT OF MULTIPLE SCLEROSIS

**(57) Abstract**

Disclosed are methods and compositions useful for the treatment of autoimmune diseases. Methods for producing vaccines against autoreactive T-cells are disclosed. The vaccines so produced are capable of restoring a degree of immunologic self-tolerance sufficient to slow or halt the progression of autoimmune disorders. In a preferred embodiment of the invention, a vaccine is derived from attenuated autologous autoreactive T-cells that recognize a variety of myelin-derived proteins. Such vaccine compositions are useful for immunologic therapy for the treatment of multiple sclerosis (MS).

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T-CELL VACCINATION FOR THE TREATMENT OF MULTIPLE SCLEROSIS

BACKGROUND OF THE INVENTION

Field of the Invention

5 The present invention relates generally to the field of immunotherapy and to treatments for autoimmune diseases. In particular, the invention relates to methods of using T-cells as vaccines for treating autoimmune diseases, including multiple sclerosis.

Description of the Related Art

10 Autoimmune diseases affect 5-7% of the adult population in Europe and North America. (Sinha AA, MT Lopez, *et al.* (1990) *Science* **248**:1380-1387). This group of diseases has a major socioeconomic impact, not only because they are accompanied by long life expectancies, but also because they strike individuals in their most productive years. For example, the patients who get multiple sclerosis
15 (MS) are predominantly women between the ages of 18 and 40.

 Autoimmune diseases are thought to result from an uncontrolled immune response directed against self antigens. In contrast, individuals who do not mount an autoimmune response to self antigens are thought to have control over these responses and are believed to be "tolerant" of self antigens. Although the etiology of
20 MS remains unknown, several lines of evidence support the hypothesis that autoimmunity plays a significant role in the development of the disease (Martin R, HF McFarland, *et al.* (1992) *Annu. Rev. Immunol.* **10**:153-187). In MS, there is evidence that the uncontrolled immune response is against the white matter of the central nervous system and more particularly to myelin proteins that are located in the white
25 matter. Ultimately, the myelin sheath surrounding the axons is destroyed. This can result in paralysis, sensory deficits and visual problems. MS is characterized by a T-cell and macrophage infiltrate in the brain. Presently, the myelin proteins thought to be the target of an immune response in MS include myelin basic protein (MBP), proteolipid protein (PLP), myelin associated glycoprotein (MAG), and myelin-
30 oligodendrocyte glycoprotein (MOG). Also there is an increasing body of evidence that the T-cell receptor has extraordinary flexibility, allowing it to react to many

different proteins (Brock R, KH Wiesmuller, *et al.* (1996) *Proc. Natl. Acad. Sci. (USA)* **93**:13108-13113; Loftus DJ, Y Chen, *et al.* (1997) *J. Immunol.* **158**:3651-3658).

Further support for this concept is based on studies of experimental
5 allergic encephalomyelitis (EAE), an animal model with clinical and pathologic similarities to MS. (Alvord, *et al.* Experimental allergic encephalomyelitis. A useful model for multiple sclerosis. New York: Alan R. Liss, 1984) In both EAE and MS, myelin basic protein (MBP), proteolipid protein (PLP), and MOG are thought to be the main target antigens for autoreactive T-cells (Brostoff SW and DW Mason (1984)
10 *J. Immunol* **133**:1938-1942; Tabira and Kira, 1992). Myelin associated glycoprotein (MAG) may be important in MS but does not produce EAE in experimental models.

The autoimmune nature of MS has to be explained in relation to the epidemiology that supports a role for an environmental agent. This is presumably a virus or viruses, or other microbes. The natural history of the disease also suggests
15 that infection may trigger exacerbations in certain patients. The debate over a role for persistent infection versus recurrent infection as the instigator of autoimmune disease remains unsettled. The mechanism of virus interaction may be molecular mimicry of host protein by invading microorganisms.

Immunologic self-tolerance appears to be achieved primarily by clonal
20 deletion of autoreactive T-cells in the thymus during negative selection, and in peripheral lymphoid tissue post maturation. However, even in healthy individuals, not all autoreactive T-cells are deleted in the thymus. Autoreactive T-cells represent part of the normal T-cell repertoire and can be isolated from normal individuals without autoimmune diseases (Correale J, M McMillan, *et al.* (1995) *Neurology*
25 **45**:1370-1378). Thus, autoreactive T-cells may exist in the periphery without causing disease. This suggests that post-thymic mechanisms control autoreactive T-cells to provide protection from immunological attacks against self. A number of mechanisms are operative *in vivo* to regulate autoreactive T-cells. Such mechanisms may involve antigen-directed T-cell clonal anergy or regulatory cellular networks that
30 influence autoreactive T-cells by interacting with their idiotypes or structures of their state of activation ergotype (Lohse AW, F Mor, *et al.* (1989) *Science* **244**:820-822;

Ben-Nun A, H Wekerle, and IR Cohen (1981) *Nature* **292**:60-61; Holoshitz J, Y Naparstek, *et al.* (1983) *Science* **219**:56-58; and Maron R, R Zerubavel, *et al.* (1983) *J. Immunol.* **131**:2316-2322). The mechanisms regarding the signaling molecules on target T-cells that elicit the idiotypic interactions are still not understood, but are
5 thought to involve both CDR2 and CDR3 hypervariable regions of the T-cell receptor V β chain (Saruhan-Direskendi G, F Weber, *et al.* (1993) *Eur. J. Immunol.* **23**:530-536). In patients with MS, resistance of T-cells to a variety of regulatory controls may account for the entry of autoimmune diseases into a chronic progressive phase (Correale J, W Gilmore, *et al.* (1996) *Nature Medicine* **2**:1354-1360). Several factors
10 make treatment of MS particularly difficult. For example, the patient's aberrant immune response to new myelin antigens expands during the period the patient appears to be in remission (Correale J, M McMillan, *et al.* (1995) *Neurology* **45**:1370-1378). In addition, in chronic MS, in contrast to acute disease, the T-lymphocytes are able to present antigen to themselves without a true antigen-presenting cell, thus
15 further amplifying the abnormal response to myelin proteins (Correale J, W Gilmore, *et al.* (1995) *J. Immunol.* **154**:2959-2968).

The course of MS is highly variable. Most typically, the disease is characterized by a relapsing pattern of acute exacerbations followed by periods of stability (remissions). However, in many cases this pattern evolves after some years
20 into a secondary progressive course, in which the clinical condition slowly deteriorates. Moreover, in some patients the disease is relentlessly progressive from the onset (primary progressive MS).

The goal of immunologic therapy is to restore tolerance without suppressing the entire immune system and causing complications such as
25 opportunistic infection, hemorrhage, and cancer. A variety of therapeutic approaches are now available in humans. These include general cytotoxic agents (cytoxan) that lack selectivity. Other examples include cyclosporin, and FK 506, that work on the cytokine IL-2 and its receptor; radiation, which induces apoptosis and cell death; corticosteroids; blockade of the MHC that prevents antigen binding; blockade of the
30 invariant TCR-CD3 complex; blockade of proinflammatory cytokines and their receptors such as IL-2 and gamma interferon and anti inflammatory cytokines such as

beta interferon; anti adhesion molecules such as CD2 or LFA; anti T-cell activation by antibody to CD4; and use of anti inflammatory cytokines such as IL-10, TGF- β and IL-4. All of these treatments are antigen non-specific and therefore cannot differentiate physiologic from pathologic responses.

5 Suppression of the immune system in a more specific way is more desirable for control of the response to self antigens without down-regulating the entire immune system. Several specific immunotherapies have been hypothesized and tested in recent years, many of which are impractical or do not work in humans. For example, high affinity peptides can be synthesized which interact with MHC class II
10 molecules and prevent the binding of encephalitogenic peptides, thereby preventing the activation of pathogenic T-cells (A Franco *et al.* (1994) The Immunologist 2:97-102). This approach is disadvantageous in that it is difficult to obtain effective concentrations of inhibitor peptides *in vivo* (Ishioka GY, L Adorini, *et al.* (1994) *J. Immunol.* 152:4310-4319). In an alternate strategy, peptides which are analogs of
15 encephalitogenic sequences (altered peptide ligands) have been shown to antagonize the T-cell receptors of antigen-specific T-cells, rendering them unreactive, although the exact mechanism is at present unknown (Jameson SC, FR Carbone, *et al.* (1993) *J. Exp. Med.* 177:1541-1550; Karin N, DJ Mitchell, *et al.* (1994) *J. Exp. Med.* 180:2227-2237; and Kuchroo VK, JM Greer, *et al.* (1994) *J. Immunol.* 153:3326-3336). Oral
20 administration of myelin has been tested and found in EAE to induce a state of immunological unresponsiveness thought to be mediated by the induction of a suppresser T-cell or of anergy (Weiner HL, A Friedman, *et al.* (1994) *Annu. Rev. Immunol.* 12:809-837; Whitacre CC, IE Gienapp, *et al.* (1991) *J. Immunol.* 147:2155-2163; and Khoury SJ, WW Hancock, *et al.* (1992) *J. Exp. Med.* 176:1355-1364). This
25 treatment has been found to be efficacious for some but not all individuals (Weiner HL, GA Mackin, *et al.* (1993) *Science* 259:1321-1324). A most recent large phase II/III trial has not shown efficacy in remitting/relapsing MS (unpublished results).

 Some studies have focused on the antigen or the T-cell that is producing the damage. For example, studies have shown that pathogenic T-cells
30 capable of inducing autoimmune diseases in animal models can be rendered "avirulent" by attenuation and can be administered as vaccines to prevent subsequent

indication of the disease (Cohen IR (1989) *Cold Spring Harbor Symposia on Quantitative Biology* 54:879-884). In these studies, T-cell vaccination induced effective anti-idiotypic and anti-ergotypic T responses. Recent studies have shown that T-cell vaccination with the avirulent cells in primates and humans afflicted with rheumatoid arthritis and MS is technically feasible and non-toxic. It also has been shown that it is possible to target and deplete a population of autoreactive T-cells involved in the autoimmune process using T-cell vaccination. Results, however, were not definitive (Hafler DA, Cohen IR, *et al.* (1992) *Clin. Immunol. and Immunopathol* 62:307-313; Lohse AW, NPM Bukker, *et al.* (1993) *J. Autoimmunity* 6:121-130; van Laar JM, AMM Miltenburg, *et al.* (1993) *J. Autoimmunity* 6:159-167; and Zhang J, R Medaer, *et al.* (1993) *Science* 261:1451-1454). However, these experimental treatments for MS have targeted only myelin basic protein activated T-cells. It is highly probable that MBP-reactive T-cells represent only a small group of the autoreactive T-cells responsible for the progression of the disease.

There currently is no effective therapy for primary or secondary MS. Thus it is evident that improvements are needed to treat MS and other autoimmune disorders with an non-toxic, effective, immunospecific approach.

THE INVENTION

Summary of the Invention

The present invention addresses the disadvantages present in the prior art. One aspect of the invention is a vaccine for the treatment of MS. The vaccine is comprised of attenuated T-cells. In a preferred embodiment of the invention, the T-cells in the vaccine are autologous. In another preferred embodiment of the invention, the T-cells target more than one myelin protein. Another aspect of the invention is a method of treating patients with MS by vaccinating patients with attenuated T-cells. Yet another aspect of the invention is a method of making a vaccine comprised of attenuated T-cells for the treatment of MS. In another preferred embodiment of the invention, the T-cells are cultured in the presence of a mixture of bovine myelin proteins.

Brief Description of the Drawings

Figure 1A-1D (collectively referred to herein as Figure 1) shows EDSS scores and changes in the frequencies of circulating bovine myelin-reactive T-cells.

Figure 2A-2C (collectively referred to herein as Figure 2) shows the number of interferon-gamma and interleukin-2 secreting T-cells reactive to bovine myelin proteins.

Figure 3A-3D (collectively referred to herein as Figure 3) illustrates changes in the frequencies of T-cells reactive to MBP, PLP and MOG peptides.

Figure 4 demonstrates inhibition of the proliferation of inoculates by anti-myelin reactive T-cell lines.

Figure 5 illustrates cytotoxicity of the anti-myelin reactive T-cells.

Figure 6 shows MHC restriction of anti-myelin reactive T-cells.

Detailed Description of the Invention

Definitions

Anti-ergotypic means against structures of a state of activation.

Anti-idiotypic means against the characteristics of an autoreactive T-cell.

Autoreactive means a B or T-cell that reacts against the host's own tissues.

As stated above, the present invention relates to a vaccine for the treatment of MS, methods of producing the vaccine, and methods for its use. The vaccine is comprised of attenuated T-cells that are presumed to be autoreactive. Preferably, the T-cells are obtained from the patient to be vaccinated. A further clarification of the target T-cell sequences (including sequences for T-cell receptors) recognized by anti-idiotypic and anti-ergotypic T-cells may be used to design synthetic peptides corresponding to predominant sequences characteristic of pathogenic myelin reactive T-cells. Therefore, this approach may be used to eliminate the need for autologous T-cell vaccination in which each patient needs his or her own vaccine. Preferably, T-cells are removed from the patient by leukapheresis. Pathogenic T-cells are estimated to occur at a frequency of between 1:20,000 to 1:40,000 peripheral blood mononuclear cells (PBMCs). Therefore, to effectively sample the repertoire it is necessary to obtain as many cells as possible.

Leukapheresis provides on the order of 1×10^9 T-cells. A sufficient number of autologous PBMCs must also be obtained to use as feeder cells during the growing of autoreactive T-cells for vaccine development.

Preferably, the PBMCs obtained are cultured in presence of cow
5 myelin proteins or synthetic complete human myelin proteins as they are identified and become available. The cells that respond to myelin proteins are selected and expanded. This is accomplished by culturing the cells in the presence of specific myelin antigens. The non-specific cells are lost in the process.

The cells are attenuated. Preferably, this is performed by irradiating
10 the cells at 12,000 Rads. Since these T-cells have been selected for their reactivity to myelin, they must be killed or they will attack the patient's myelin when injected. The irradiated cells are not frozen, although the fresh cells can then be stored frozen and then irradiated and used for future injection into the patient.

Patients preferably receive subcutaneous injections of attenuated T-
15 cells every 4-6 weeks. The number of cells is preferably 40,000,000. However, the optimum number of cells may vary by patient. The preferred range of cells/vaccination is between 30 and 80×10^6 . Previous T-cell vaccination protocols in multiple sclerosis and rheumatoid arthritis have used $30-60 \times 10^6$ cells/vaccination without serious side effects (van Laar JM, AMM Miltenburg, *et al.* (1993) *J. Autoimmunity* 6:159-167; Zhang J, R Medaer, *et al.* (1993) *Science* 261:1451-1454).
20 Inoculations are given in 4-6 week intervals for 6 months and depending on clinical, immunologic and MRI data, the dose and interval for the injections may be adjusted.

Preferably, if after the first 2 inoculations, patients do not respond clinically to the number of myelin autoreactive T-cells administered, a dose-escalation
25 administration is started. The number of inoculated cells may be increased 25% each 3 months to the point at which adverse reactions appeared. This type of gradual escalation can provide information on the upper limits of safety and indicate a dose range in which efficacy studies could be conducted. If no escalation is necessary, injections are given in 3 month intervals for the next 18 months.

30 Adverse reactions may be reactions such as: 1) systemic symptoms that require in-patient hospitalization; 2) a phase of increasing disability that progresses

two or more steps in EDSS scale over two consecutive scheduled neurologic evaluations; 3) CD4+ lymphocyte counts below 500 cells/mm³.

Without wishing to be bound by any particular theory, the mechanism of action for the vaccine is believed to be a host response to the T-cell receptor(TCR) variable region on the irradiated pathogenic T-cell that comprises the vaccine. This region is the only area thought to be different on the pathogenic T-cell as compared to other naive or activated T-cells. The approach described herein is based on the hypothesis that there are many V_α and V_β families involved since progressive MS has so many different antigen specific responses and immunodominant epitopes may differ from patient to patient. This allows T-cells from each patient to be activated against epitopes it has seen *in vivo*. when inactivated by radiation, the TCRs become antigens and induce either an anti-idiotypic antibody or a T-cell response against the V_α and V_β regions of many different pathogenic T-cells in that patient. The result is either down regulation or killing of existing and future pathogenic responses. Since it is a "killed" vaccine, it may be necessary to give a booster once a year to perpetuate the anti-myelin specific T-cells inactivation or killing.

Example 1

Four patients with definite secondary progressive MS and without response to any other available treatment were studied. Age range was 32-45 years, and no sex criteria was used. Progression of at least one unit in the Kurtzke scale occurred in the year prior to entry. The patients were otherwise healthy and had no other diseases to explain their neurologic conditions. All patients were free of immunotherapy for 60 days, or steroids for at least 90 days prior to the start of this protocol. Two patients had never received any prior treatment for MS.

Peripheral blood mononuclear cells (PBMCs) were obtained by leukapheresis. Approximately 10⁵ - 10⁶ myelin protein specific T-cells can be obtained per apheresis. To obtain 40 x 10⁶ cells for vaccination required a 40-400 fold expansion. Leukapheresis was performed prior to vaccination. There were 6-8 weeks between the first apheresis and the first injection.

Routine blood samples were obtained for immunologic safety by standard venipuncture. Patients were asked to donate 50-70 cc of blood at monthly

intervals for three months and then at two-months intervals for 21 months for routine assessment for safety measures and to assess the effect of the vaccination program on the immune system.

To establish autoreactive T-cell lines PBMCs were cultured in serum-free media supplemented with gentamicin and stimulated with bovine total myelin proteins prepared according to standard protocols (Correale J, M McMillan, *et al.* (1995) *Neurology* 45:1370- 1378) and sterilized by filtration through a 0.22 micron filter. After 5-7 days cells were expanded using 50 U/ml of recombinant human IL-2 (Cetus). T-cell lines were re-stimulated after 10-14 days using autologous irradiated PBMCs as antigen presenting cells (APCs) and bovine myelin proteins. Cycles of restimulation and expansion were repeated weekly until the response to myelin antigens detected in proliferation assays exceeded the response to control antigens by three fold. At that time, usually following 3-4 cycles of restimulation and expansion, activated myelin specific T-cells were separated from APCs using Ficoll gradient separation, washed in sterile phosphate buffered saline (PBS) and irradiated for attenuation (12000 rads Cs¹³⁷). Aliquots of living cells were frozen (prior to irradiation) for future injections administered every 6-12 weeks. For these injections, cells were thawed and then irradiated just prior to inoculation.

Each patient received 40×10^6 cells resuspended in 1 ml of sterile PBS and injected subcutaneously (0.5 ml/arm). Prior to injection, an aliquot of the T-cell preparation was tested for bacterial growth, endotoxins, fungus, cytomegalovirus, herpes simplex, adenovirus, varicella zoster and mycoplasmas (GMP). In addition, a skin test was performed using intradermal injection of 25,000-50,000 T-cells suspended in 0.1 ml of sterile PBS to test for immediate type hypersensitivity. These procedures were repeated prior to each inoculation. The patients were kept as in-patients for the first 48 hrs. following vaccination. Vaccination was repeated at 3-month intervals for the first two patients and at 6-week intervals for the second two patients for 6 months, and then all 4 patients were vaccinated at 3-month intervals.

Patients were monitored to determine whether there was any progression or improvement of neurologic deficits and neuropsychological profile. Neurological progression was defined as an increase of one or more EDSS steps

maintained for more than 90 days. All patients had a baseline and annual MRI study (brain or spinal cord, according to localization of the lesions) at month 12 and month 24 after vaccination. A reduction in lesions area and in percentage change in lesions area from baseline to one year and two years for individual subjects were used as parameters for efficacy. Patients should have MRIs done at 3-month intervals to check efficacy and determine dose and frequency of the vaccine. Frequency of circulating myelin-reactive T-cells before and after each inoculation was measured to determine whether a decline in such cells correlated reciprocally with the proliferative responses of peripheral blood mononuclear cells to the inoculates

10 Treatment discontinuation criteria were pregnancy, CD4+ lymphocyte counts below 500 cells/mm³, occurrence of grade III or IV toxicity, use of other investigational or experimental therapies of MS, a phase of increasing disability that progresses two or more steps in the EDSS scale unremittingly over a six month period, or serious intercurrent illness precluding continued treatment with T-cell

15 vaccine.

Measurement of Immunological Response

T-cell response to the inoculates was examined in PBMC by using a standard 60 hr. proliferation assay, and the responses were compared with T-cell blasts prepared concurrently by PHA stimulation and resting autoreactive myelin T-cells (8-10 days after the last stimulation with antigen presenting cells and myelin).

20 Frequency of T-cells capable of suppressing the proliferation of inoculates was measured using frequency analysis. Cultures exerting more than 60% inhibition on the proliferation of inoculates were considered as responding T-cell lines. Anti-idiotypic and anti-ergotypic responses were evaluated using standard Cr⁵¹ release

25 assays. Patterns of cytokine secretion of anti-idiotypic, anti-ergotypic and myelin reactive T-cells were evaluated by ELISAs and ELISPOTs. Phenotyping of the regulatory populations and fresh PBMC was studied using flow cytometry analysis.

Results

We have recently isolated anti-idiotypic and anti-ergotypic T-cells by

30 *in vitro* stimulation of T-cells with autologous irradiated autoreactive PLP CD4+ T-

cells. These anti-idiotypic and anti-ergotypic T-cell clones express a CD8+ phenotype and lyse *in vitro* auto-reactive CD4+ PLP T-cell clones.

Following vaccination, three of the patients tested had no change in EDSS score in follow up testing conducted for 414 days (patient ML), 326 days (patient MK), or 171 days (patient GL). In addition, the patients had no T-cell response to bovine myelin (Figure 1). In these three patients, we observed a decrease in the number of interferon-gamma (IFN-gamma) and interleukin-2 (IL-2) secreting T-cells reactive to bovine myelin proteins (Figure 2). In all four patients, dramatic decreases in the frequencies of T-cells reactive to MBP, PLP and MOG peptides also was observed (Figure 3). Figure 4 demonstrates inhibition of the proliferation of the inoculates by anti-myelin reactive T-cell lines. Figure 5 shows the cytotoxicity of the anti-myelin reactive T-cells. Figure 6 shows MHC restriction of anti-myelin reactive T-cells.

In conclusion, our previous data support the notion that T-cells from chronic progressive MS patients are resistant to a variety of immunosuppressive mechanisms, as well as Immunomodulatory drugs phase (Correale J., W. Gilmore, *et al.* (1996) *Nature Medicine* 2:1354-1360). These findings represent the rationale to develop new alternatives for the treatment of progressive MS. The broad-based immune response in this group of patients requires the widest range of antigen-specific T-cells to be inactivated. This therapeutic approach is a unique T-cell vaccine and is described in the protocol.

The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

CLAIMS

What is Claimed is:

- 1 1. A vaccine comprising, in an amount effective to suppress an
2 autoimmune disorder upon administration to a human, attenuated T-cells.
- 1 2. The vaccine of claim 1, wherein the autoimmune disorder is multiple
2 sclerosis.
- 1 3. The vaccine of claim 2, comprising T-cells cultured in the presence of
2 natural or synthetic myelin proteins.
- 1 4. The vaccine of claim 3, wherein the vaccine is prepared by selecting
2 and expanding T-cells that respond to myelin proteins.
- 1 5. The vaccine of claim 1, wherein the T-cells are derived from
2 autologous peripheral mononuclear cells.
- 1 6. The vaccine of claim 1, wherein the T-cells are attenuated by
2 irradiation.
- 1 7. The vaccine of claim 5, wherein the cultured, attenuated T-cells are
2 frozen before attenuation.
- 1 8. A method of mediating an immune response, comprising the step of
2 administering attenuated T-cells to a human.
- 1 9. The method of claim 8, wherein the T-cells are derived from
2 autologous peripheral mononuclear cells.
- 1 10. The method of claim 8, wherein the T-cells comprise T-cells cultured
2 in the presence of natural or synthetic myelin proteins.
- 1 11. The method of claim 10, wherein the T-cells are prepared by selecting
2 and expanding T-cells that respond to myelin proteins.
- 1 12. The method of claim 8, wherein the attenuated T-cells are attenuated
2 by irradiation.
- 1 13. The method of claim 8, wherein the T-cells target more than one
2 myelin protein.
- 1 14. The method of claim 8, wherein the T-cells are administered
2 subcutaneously.

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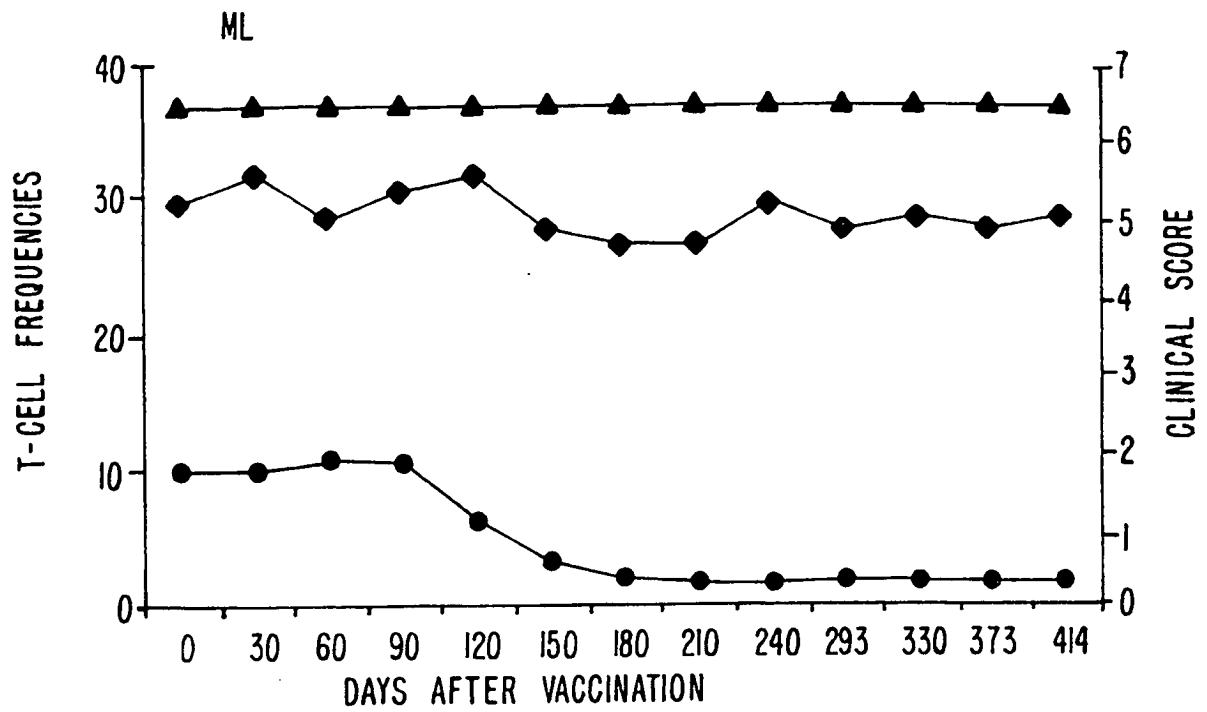


FIG. 1A.

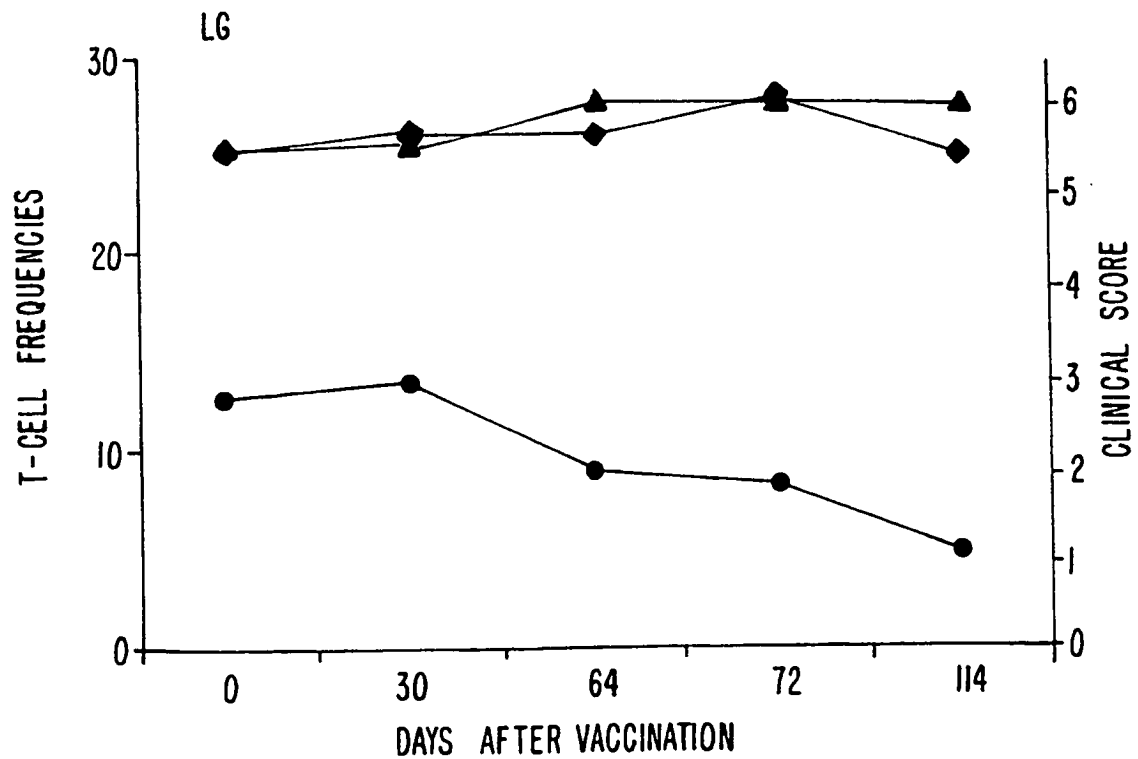


FIG. 1B.

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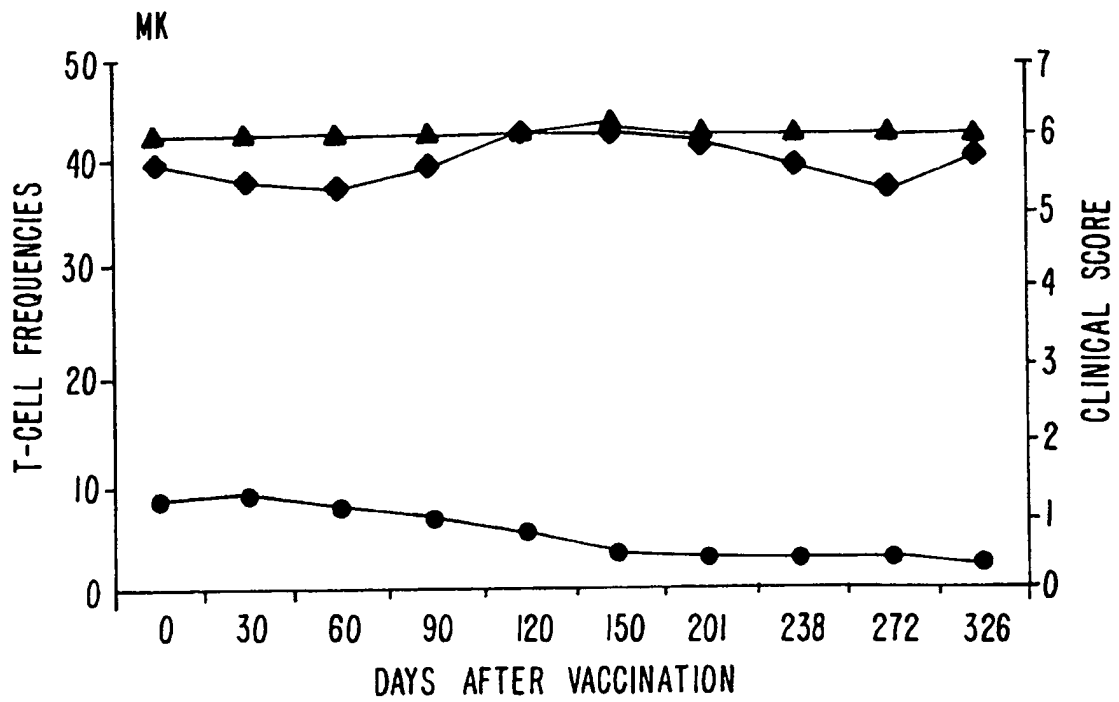


FIG. 1C.

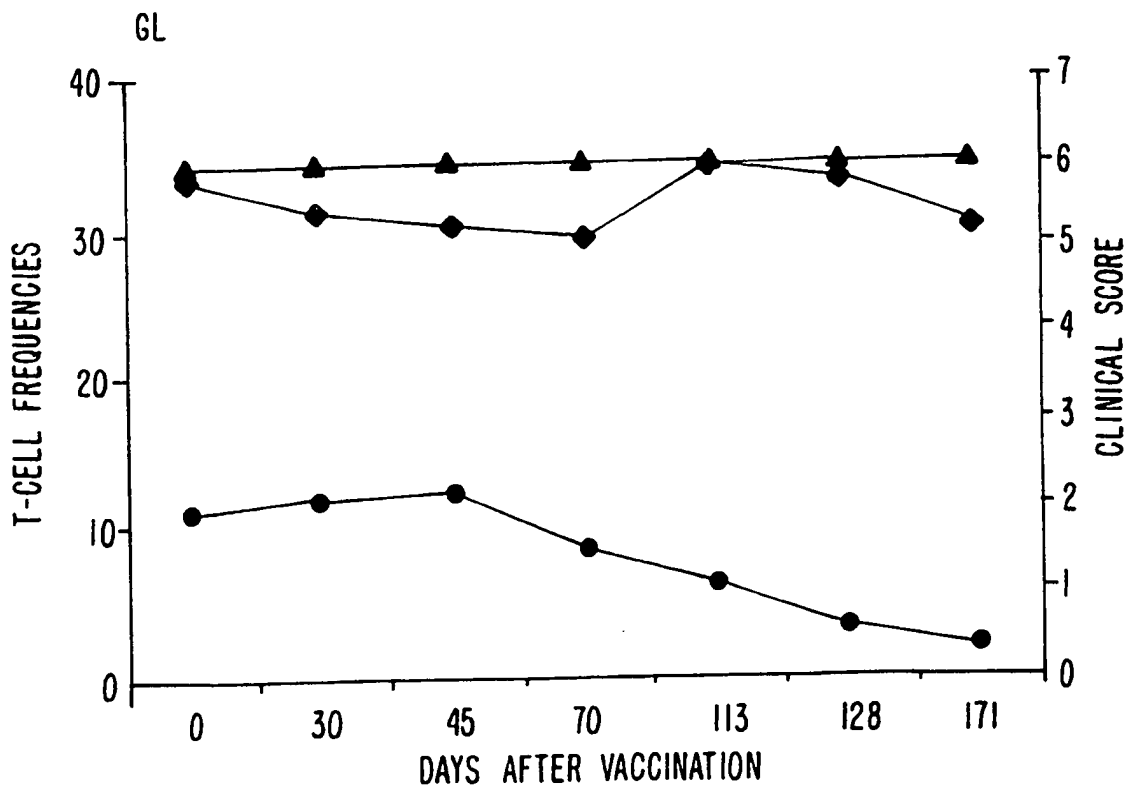
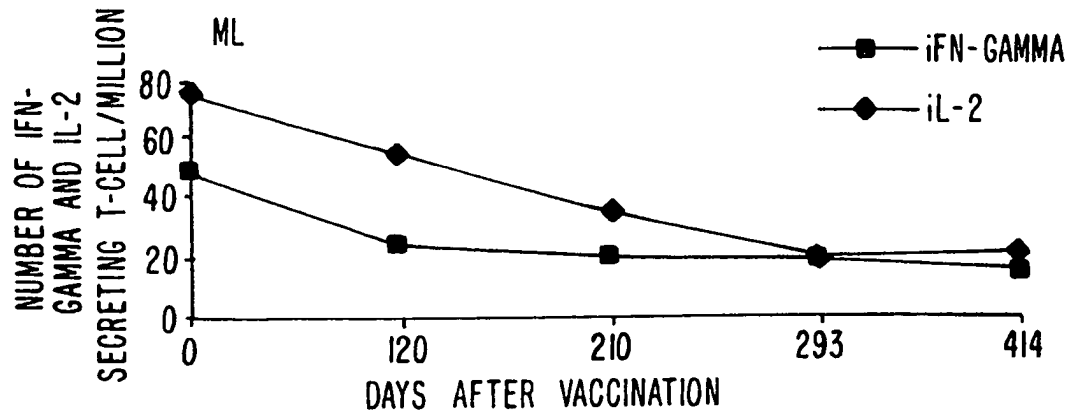
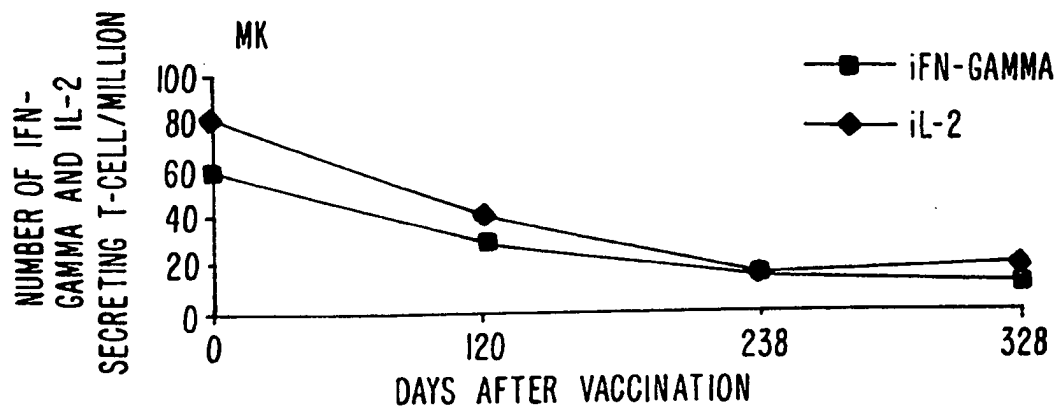
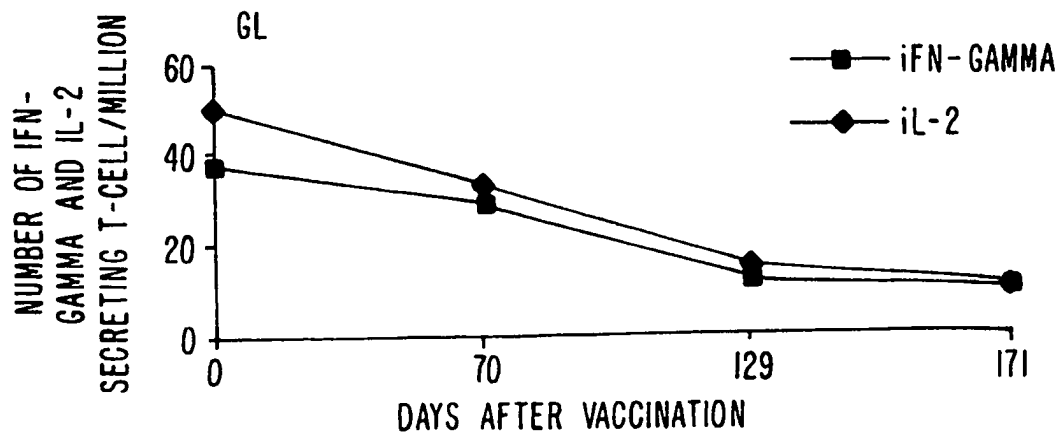
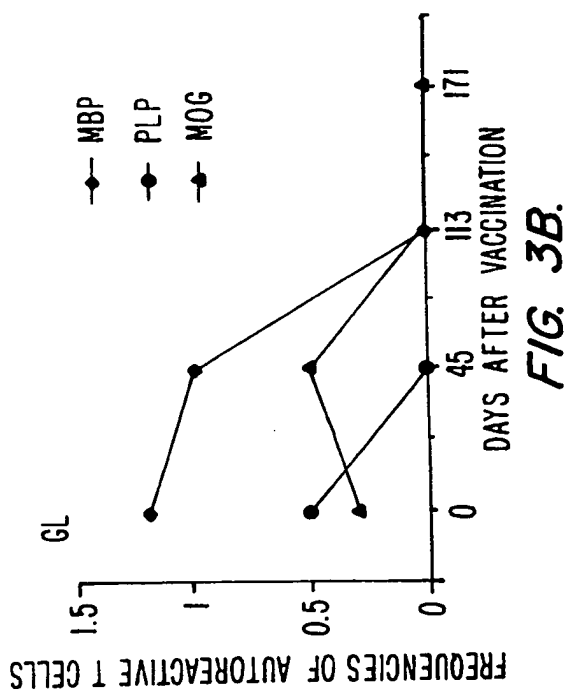
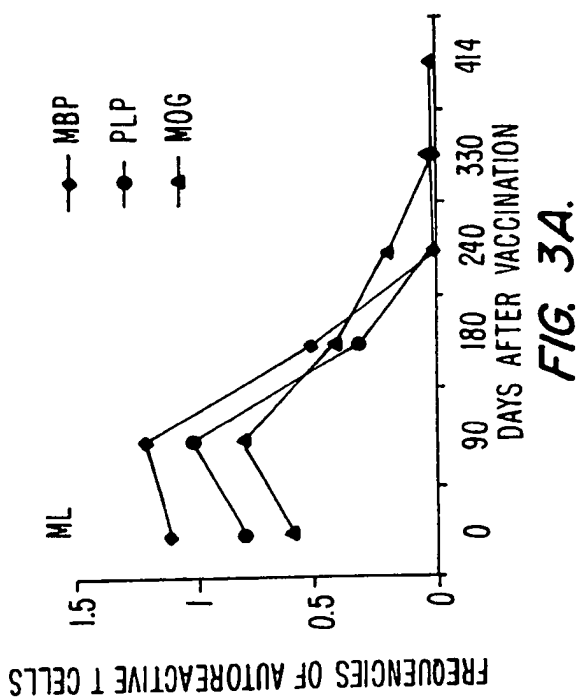
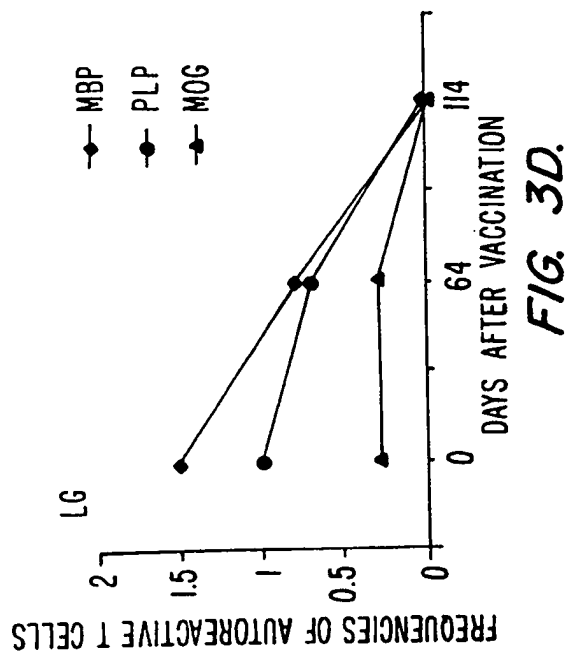
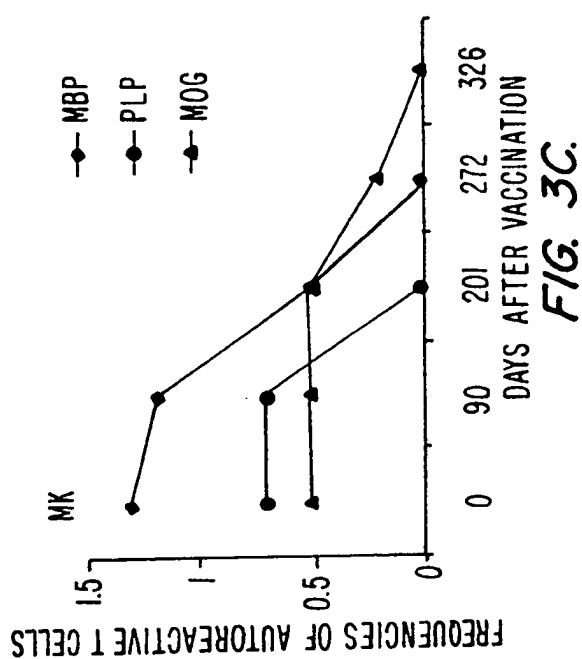


FIG. 1D.

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*FIG. 2A.**FIG. 2B.**FIG. 2C.*



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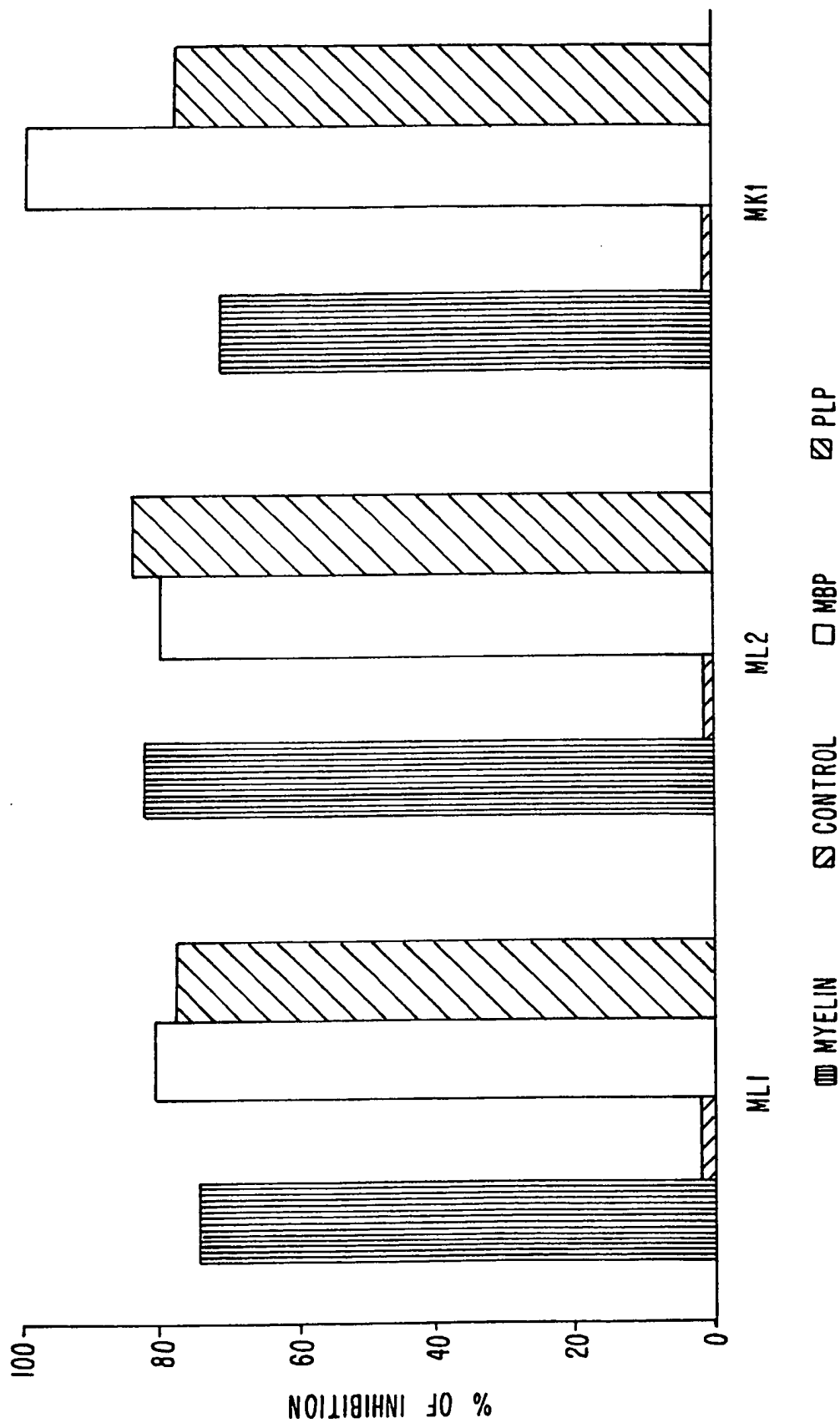


FIG. 4.

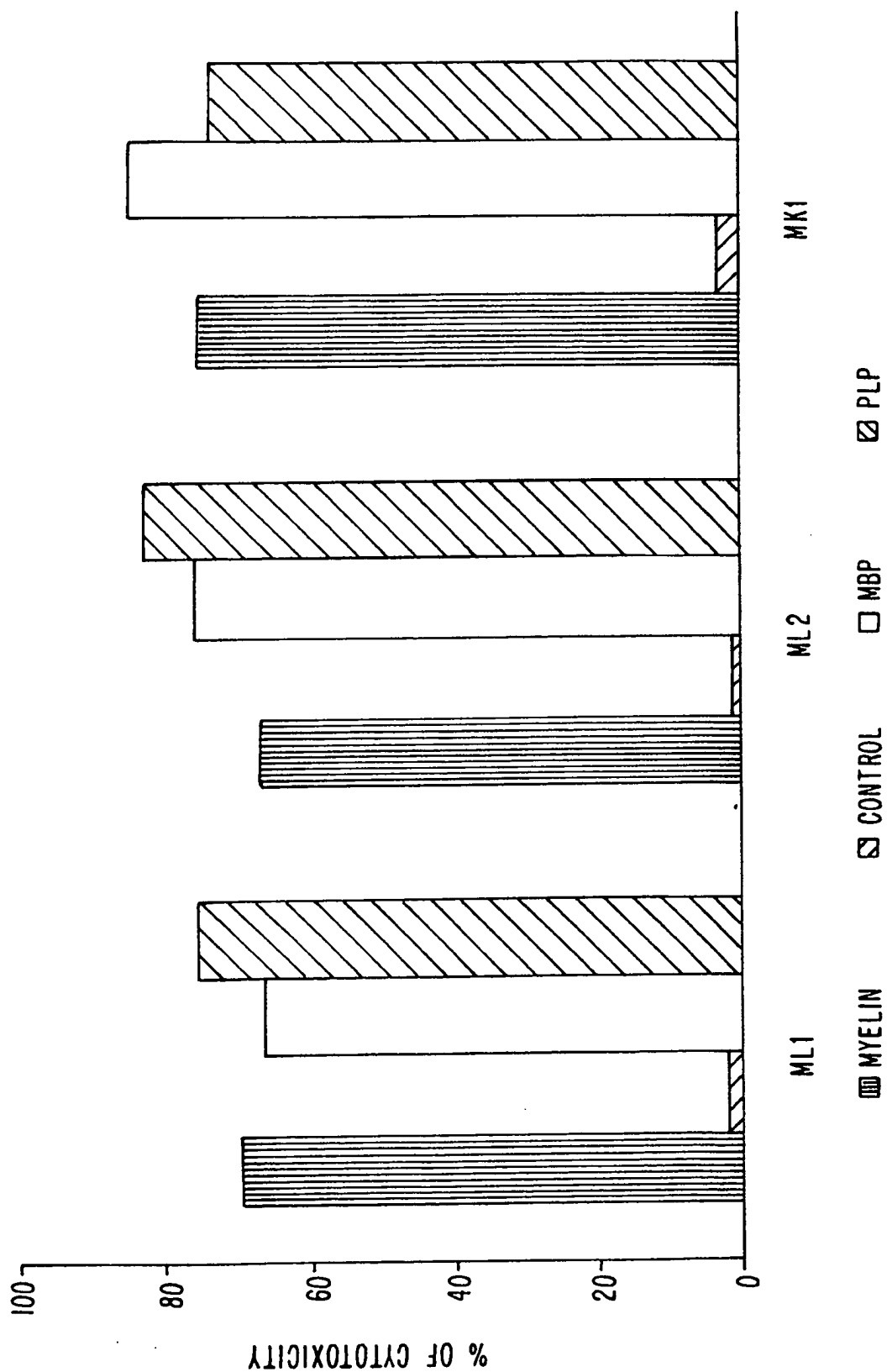


FIG. 5.

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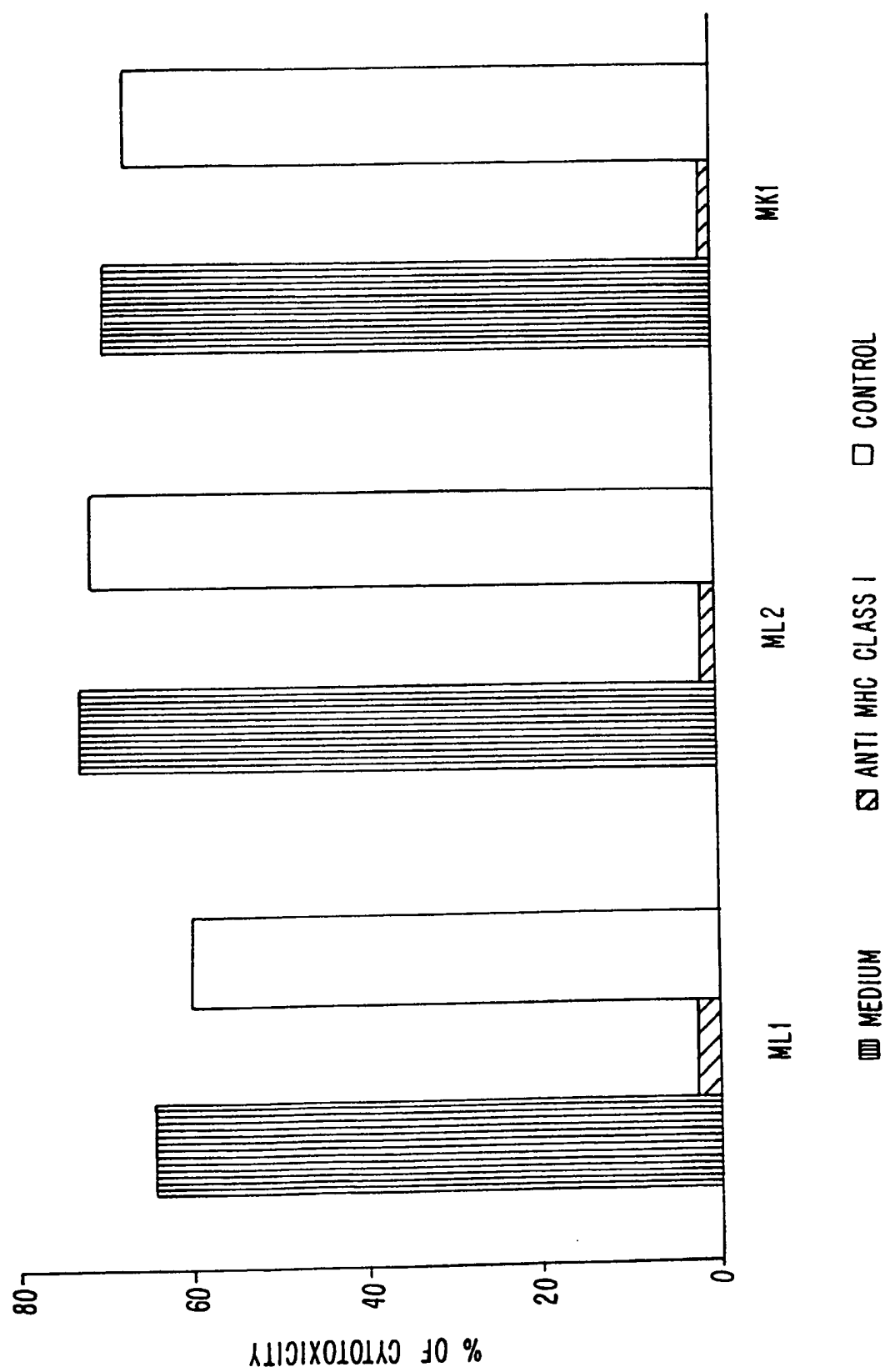


FIG. 6.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K39/00 //A61K35/14,C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 15225 A (BRIGHAM AND WOMEN'S HOSPITAL ET AL.) 17 October 1991 see page 12, line 26 - page 13, line 4 see claims ---	1-20
X	J. ZHANG ET AL.: "MHC-restricted depletion of human myelin basic protein-reactive T cells by T cell vaccination." SCIENCE, vol. 261, no. 5127, 10 September 1993, pages 1451-1454, XP002091570 Washington, DC, USA cited in the application see the whole document --- -/--	1-20



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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- "&" document member of the same patent family

Date of the actual completion of the international search

28 January 1999

Date of mailing of the international search report

16/02/1999

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	J. ZHANG ET AL.: "Myelin basic protein-reactive T cells in multiple sclerosis: Pathologic relevance and therapeutic targeting." CYTOTECHNOLOGY, vol. 16, no. 3, 1994, pages 181-187, XP002091571 Dordrecht, The Netherlands see the whole document ---	1-20
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X	J. CORREALE ET AL.: "Isolation and characterization of CD8+ T-cell clones (TCCs) that recognize autologous-activated CD4+ TCCs from chronic progressive multiple sclerosis (MS) patients: Implications for immunoregulation in MS." NEUROLOGY, vol. 46, no. 2 suppl. S, February 1996, page A280 XP002091574 Minneapolis, MN, USA see abstract S25.006 ---	1-6,20
X	L. WEINER ET AL.: "Isolation and characterization of CD8+ and gamma-delta T cells specific for autologous autoreactive CD4+ T cell clones from multiple sclerosis patients and healthy control subjects." NEUROLOGY, vol. 48, no. 3 suppl. 2, August 1997, pages A59-A60, XP002091575 Minneapolis, MN, USA see abstract P01.108 ---	1-6,20
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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